Annu. Rev. Physiol. 1999. 61:19–43

CELLULAR AND MOLECULAR BASIS FOR ELECTRICAL RHYTHMICITY IN GASTROINTESTINAL MUSCLES

Burton Horowitz, Sean M. Ward, and Kenton M. Sanders
University of Nevada School of Medicine, Department of Physiology and Cell Biology, Reno, Nevada 89557; e-mail: burt@physio.unr.edu; sean@physio.unr.edu; kent@physio.unr.edu

KEY WORDS: gastrointestinal smooth muscle, K^+ channels, Cl^- channels, Ca^{2+} channels, enteric nervous system, interstitial cells of Cajal

ABSTRACT

Regulation of gastrointestinal (GI) motility is intimately coordinated with the modulation of ionic conductances expressed in GI smooth muscle and nonmuscle cells. Interstitial cells of Cajal (ICC) act as pacemaker cells and possess unique ionic conductances that trigger slow wave activity in these cells. The slow wave mechanism is an exclusive feature of ICC: Smooth muscle cells may lack the basic ionic mechanisms necessary to generate or regenerate slow waves. The molecular identification of the components for these conductances provides the foundation for a complete understanding of the ionic basis for GI motility. In addition, this information will provide a basis for the identification or development of therapeutics that might act on these channels. It is much easier to study these conductances and develop blocking drugs in expression systems than in native GI muscle cells. This review focuses on the relationship between ionic currents in native GI smooth muscle cells and ICC and their molecular counterparts.

INTRODUCTION

The contractile behavior of gastrointestinal (GI) smooth muscles depends to a considerable extent on the intrinsic electrical activities of the muscles. This is particularly true of the phasic portions of the GI tract where cyclic

0066-4278/99/0315-0019$08.00

depolarizations and repolarizations, referred to as slow waves, determine contractile frequency and maintain the phasic nature of contractions. The slow wave cycle insures a period of relaxation between contractions to allow mixing and movement of lumenal contents. Tonic regions of the GI tract, such as the sphincters and gastric fundus, are also regulated by electrical events but primarily by more persistent changes in membrane potential due to stimulation by neurotransmitters or hormones.

GI smooth muscles exhibit a wide range of electrical behaviors (see 1), and understanding the mechanisms of these events has been the goal of physiologists for more than half a century. Electrical activity can vary from slow changes in membrane potential, to hyperpolarization and depolarization responses to neurotransmitters, to oscillatory slow wave activity, to fast Ca^{2+} action potentials. All this behavior can be recorded during impalements of a single smooth muscle cell, which suggests that a plethora of ionic conductances and regulatory mechanisms are at play in GI muscles. Such diversity is almost unprecedented in other excitable cells. Diversity, the small size of smooth muscle cells, and the structural complexities of GI muscles have slowed progress toward understanding the ionic basis for electrical rhythmicity.

Since the mid-1980s, a considerable amount of information about the ionic mechanisms responsible for GI electrophysiology has been provided by application of the patch clamp technique (see 2, 3) and molecular techniques (e.g. 4, 5). Investigations into these areas are beginning to break apart the complexities of GI electrical rhythmicity. We can now attribute some of the events observed to specific cell types, particular ionic conductances, and specific molecular species of channels. Identification of the molecular components responsible for the ionic conductances found in GI smooth muscles is allowing studies of the biophysical and regulatory characteristics of these channels in expression systems devoid of contaminating currents. Assignment of molecular entities to the ionic events underlying GI electrical activity may allow these channels to become targets for therapeutic agents, because it is much easier to study the properties of these conductances and to test blocking drugs in expression systems than in native GI muscle cells. This review focuses on current knowledge of the cellular and molecular basis for electrical rhythmicity and the ionic species that have been identified in GI smooth muscles.

ORGANIZATION OF ELECTRICAL ACTIVITY IN GI MUSCLES

Understanding the electrical activity of the GI tract is complicated by the following. (*a*) The electrical output of GI muscles is a product of contributions from two electrically coupled cell types, smooth muscle cells and interstitial cells of

Cajal (ICC) (for review, see 6). These cells have distinct electrical missions and express different types of ionic conductances to accomplish those tasks. ICC serve as pacemaker cells and generate and propagate electrical slow waves. Smooth muscle cells respond to the depolarization/repolarization cycle imposed by ICC. The responses of smooth muscle cells are focused on the regulation of L-type Ca^{2+} current, which is the main source of Ca^{2+} for contraction. (*b*) Regulatory input from nerves, hormones, and paracrine substances are superimposed upon the ongoing myogenic activity. Responses to biologically active substances result from modulations of ionic conductances that are already active and going through dynamic changes in open probability during the slow wave cycle and from activation of new conductances that do not participate in basal electrical activity. The conductances affected by regulatory substances could be expressed in either smooth muscle cells or ICC. Finally, the conductance of both cell types mutually affects the electrical behavior of the total syncytium. Electrical responses from such a complex array of electrically coupled cells are not easily predictable from studies on isolated myocytes. Therefore, studies of GI electrophysiology must include detailed investigations of both cell types and of intact muscles to understand basic mechanisms and integrated responses.

Before we can comprehend the significance of the molecular diversity that generates the electrical behavior of GI muscles, the morphological features of GI muscles and the general organization of electrical activity must be understood. For years, smooth muscles of the GI tract were considered homogenous populations of cells within a given organ. Now we recognize that important regional differences exist: The activities of the circular and longitudinal muscle layers of a given organ can vary widely, and the activities of smooth muscle cells within a muscle layer can be profoundly different (7–10). This suggests region-by-region fine control of the development and organization of ionic conductances in GI muscles, and developmental studies have documented changes in K^+ currents as electrical activity develops (11). The developmental influences and cell biology that ultimately determine the unique characteristics of electrical activity in a given region of muscle are poorly understood. These questions are of great importance if we are to understand how electrical diversity is established and maintained in GI muscles.

Role of ICC in Electrical Rhythmicity

As stated above, a major source of regional electrical diversity arises from the contributions of ICC. These cells populate all pacemaker regions in the GI tract (see 6, 12–14), and ICC lie at the interface between varicose nerve fibers and smooth muscle cells (e.g. 15). ICC are electrically coupled to the smooth muscle syncytium via gap junctions. Therefore, expression of ionic conductances in ICC can influence the resting potential, electrical activity, and

responses of the coupled smooth muscle cells. By the same reasoning, the electrical activity of smooth muscle cells may influence the output of ICC. There is not room in this review to cover the anatomy and physiology of ICC in depth. For more information the reader is directed to recent reviews (6, 16).

ICC are pacemaker cells that generate and conduct electrical slow waves to smooth muscle cells (17–20). Other types of ICC receive, transduce, and conduct neural signals to the smooth muscle (21). Therefore, the search for the ionic mechanisms that produce and regulate electrical activity in the GI tract must be expanded to include systematic investigation of ICC. We know little about the unique ionic conductances expressed by ICC.

Most investigators have envisioned ICC to be pacemaker cells like those in the heart. It has been thought that ICC generate pacemaker current that depolarizes smooth muscle cells to threshold. Slow waves were thought to be regenerated or even amplified by the smooth muscle (22). In this concept, ICC are the pacemakers because they are more excitable than smooth muscle cells. Numerous studies have shown that loss of ICC from GI muscles blocks generation of spontaneous slow waves (18–20, 23–25). If smooth muscle has the ability to regenerate and actively propagate slow waves, then in preparations lacking ICC it should be possible to evoke slow waves with an external current source. Several observations do not support this hypothesis (see Figure 1): (*a*) Slow waves passively decay as a function of distance from pacemaker areas (see 9); (*b*) removal of the pacemaker from part of a muscle strip causes slow waves to passively decay through the region lacking ICC (25, 26); (*c*) after removal of ICC, smooth muscle cells continue to be excitable, but in the absence of ICC, smooth muscles produce action potentials rather than slow wave-like activity (19, 27); and (*d*) after removing ICC, it is not possible to evoke slow waves by electrical pacing. Taken together, these observations suggest a novel hypothesis (Figure 2): The slow wave mechanism is an exclusive feature of ICC; smooth muscle cells lack the basic ionic mechanisms necessary to generate or regenerate slow waves. A corollary to this hypothesis is that active propagation of slow waves occurs through networks of ICC instead of being amplified and propagated by smooth muscle cells (e.g. 22).

Conduction of slow waves from ICC into smooth muscle cells is passive. Microelectrode impalements of smooth muscle cells record slow waves that have been conducted through gap junctions from ICC. The depolarization caused by slow waves activates different populations of ionic conductances in smooth muscle cells, most importantly L-type Ca^{2+} channels. The smooth muscle response may be manifest as a small, nonregenerative enhancement in depolarization (i.e. an apparent increase in slow wave amplitude) (see Figure 1*E*) or Ca^{2+} action potentials superimposed upon the slow waves (also see Figure 1*E*). The response of smooth muscle cells to slow wave depolarizations is determined by the magnitude of the depolarization, the passive properties of the smooth

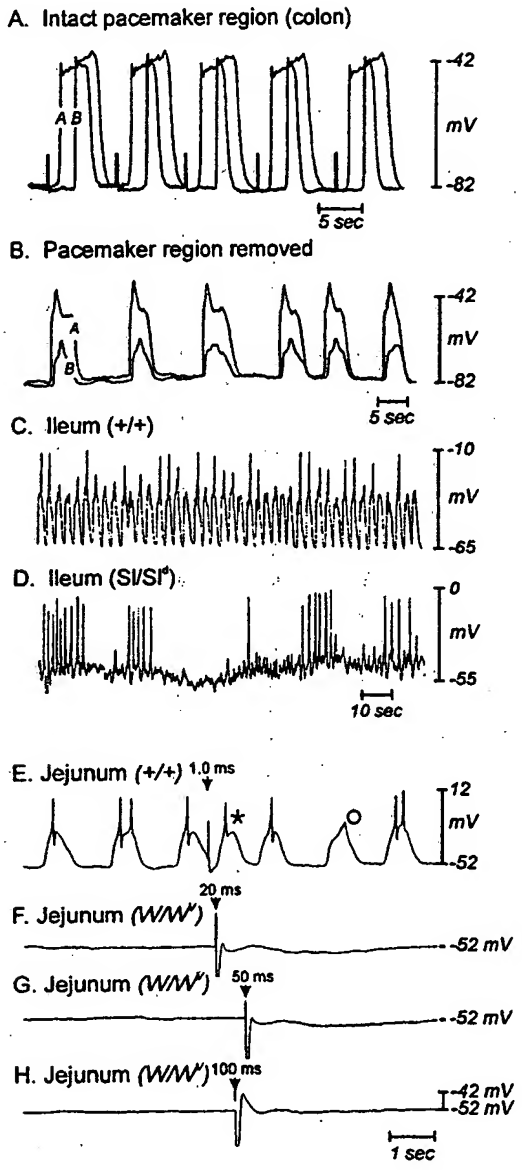
muscle syncytium, and the particular ionic conductances available. If threshold is reached, Ca^{2+} action potentials are generated. These events can propagate for short distances, but the impedance properties of smooth muscle are such that the propagation distance is limited (see 28).

This organization allows slow waves to spread actively through ICC networks within GI muscles at all times, but coupling of slow waves to contractions can be regulated by external stimuli, such as neural inputs. In regions of cells where nonselective cation channels are activated (primary electrical mechanism utilized by excitatory neurotransmitters), slow waves are superimposed upon the depolarization, and the open probability of Ca^{2+} channels is enhanced. In regions in which the open probability of K^+ channels is enhanced (primary mechanism utilized by inhibitory transmitters), slow waves fail to produce adequate depolarization to activate sufficient numbers of Ca^{2+} channels to yield contractions. The ionic conductances activated by neurotransmitters and other agents may reside in smooth muscle cells or ICC, but activation or suppression of conductances influences the behavior of the total syncytium. This hypothesis substantially alters the old concept of the myogenic control of motility and provides a new framework for the design of studies into the molecular components of excitability. We must reduce GI muscles to smooth muscle cells and ICC and examine both for the ionic conductances that contribute to the behaviors of GI muscles.

Ionic Conductances Unique to ICC

We know little about the molecular nature of ionic conductances in ICC. ICC were first isolated in 1989 (see 17); however, work has progressed slowly on these cells because of the difficulties in isolating and identifying the cells. ICC from the canine colon pacemaker region express ionic conductances unique from smooth muscle cells isolated from the same region (29). Both cell types express 4-aminopyridine–sensitive delayed rectifier currents, but the half inactivation of the current in ICC occurred 25 mV more negative than the current found in circular smooth muscle cells from the pacemaker region. An inward current was observed in interstitial cells that had a resolution threshold of −70 mV. Activation of this current occurred at least 20 mV negative to the potential at which inward currents could be resolved in smooth muscle cells. The negatively activating inward current in ICC was carried by Ca^{2+} and had properties of a low-threshold Ca^{2+} current similar to that found in the SA node of the heart (30). Recent experiments have also detected a prominent Ba^{2+}-sensitive inward rectifier current in pacemaker ICC from the canine colon (E Flynn, SD Koh, KM Sanders, unpublished studies). This conductance may contribute to the negative resting potentials in the pacemaker area (−78 mV) (see 9).

The inward current source for pacemaker activity appears to vary in other regions of the GI tract. Recent studies on primary cultures of ICC from the


A. Intact pacemaker region (colon)
A B
-42
mV
-82
5 sec
B. Pacemaker region removed
A
B
-42
mV
-82
5 sec
C. Ileum (+/+)
-10
mV
-65
D. Ileum (Sl/Sl^{w})
0
mV
-55
10 sec
E. Jejunum (+/+) 1.0 ms
12
mV
-52
F. Jejunum (W/W^{v}) 20 ms
-52 mV
G. Jejunum (W/W^{v}) 50 ms
-52 mV
H. Jejunum (W/W^{v}) 100 ms
-42 mV
-52 mV
1 sec

murine small bowel suggest that a Ca^{2+}-dependent Cl^- current might contribute to pacemaker activity in that region (31). Cultured small bowel ICC exhibited rhythmic inward currents that were blocked by intracellular EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid], intracellular solutions nominally free of Ca^{2+}, and extracellular 4-acetoamido-4-isothiocyanatostilbene-2,2'-disulphonic acid (SITS). The rhythmic currents reversed at potentials close to the equilibrium potential for Cl^-. Currently, it is difficult to reconcile these findings with the mechanism of slow wave in vivo because studies of intact murine small intestine have shown that blockers of Ca^{2+}-activated Cl^- currents, such as niflumic acid (SM Ward, unpublished observations), or replacement of most extracellular Cl^- with isethionate (32) did not inhibit slow waves. Recent voltage clamp studies of cultured ICC from murine intestine have suggested a nonselective cation conductance might participate in spontaneous rhythmicity (SD Koh, KM Sanders & SM Ward, unpublished observations). Removal of Na^+ from the extracellular solution, however, reduced, but did not

Figure 1 Slow waves are generated and propagate within interstitial cells of Cajal (ICC) networks in gastrointestinal muscles. *Panels A* and *B* show the need for ICC for active propagation of slow waves. *Panel A* shows records from a dual microelectrode impalement of cells along the submucosal surface of the canine colon. (*electrodes A* and *B*). Impaled cells 11 mm apart (records are denoted). A stimulator paced the muscle strip. Slow waves were evoked at a constant rate (note stimulus artifacts) and propagate with fixed conduction velocity. At all points along the submucosal surface slow waves were of approximately equal amplitude, demonstrating active propagation. *Panel B* shows a similar experiment, but a thin strip of tissue along the submucosal surface containing the pacemaker cells was removed from most of the strip. The small bit of submucosal surface remaining generated normal slow waves. These events decayed in amplitude as a function of distance from the pacemaker area. *Panel B*, *A* and *B* show simultaneous intracellular recordings made from cells 6 mm apart. [Data in both panels redrawn from Sanders et al (136).] *Panel C* shows normal electrical activity from the wild-type (+/+) mouse ileum. Spontaneous slow waves elicited spike potentials that were superimposed upon the slow waves. *Panel D* shows activity in a stem cell factor mutant (Sl/Sld) in which myenteric ICC (IC-MY; pacemaker cells) fail to develop. Slow waves are not generated in these tissues. However, some muscles generate spontaneous action potentials (as shown), and these events are blocked by nifedipine (1 μM) (not shown). Although the circular muscle depolarizes and produces spontaneous activity in the form of action potentials, slow wave type rhythmicity is never observed in these muscles. This suggests that the ionic mechanism responsible for slow waves is not present in ileal smooth muscle cells. [Data in *Panels C* and *D* redrawn from Ward et al (27).] *Panels E–H* show recordings from the jejunums of wild-type and a c-Kit mutant (W/WV). IC-MY also fail to develop in W/WV animals (18). In +/+ animals, spontaneous slow waves are apparent with superimposed spike activity (*Panel E*). Premature slow waves (*) can be evoked with electrical field stimulation with pulse durations as short as 1 ms (*arrow*). The slow wave (denoted by the *circle*) failed to reach threshold. Spontaneous slow waves were not recorded from muscles of W/WV animals, and electrical field stimulation (1–100 ms) was unable to elicit slow waves (*Panels F–H*) even with long-duration pulses. *Panels E–H* show experiments performed in the presence of tetrodotoxin (1 μM) to block the influence of intrinsic nerves. (*Panel H*) Time base is applicable to *Panels E–H*. These data suggest that slow waves are an exclusive property of ICC, and they are not amplified or regenerated in smooth muscle cells.

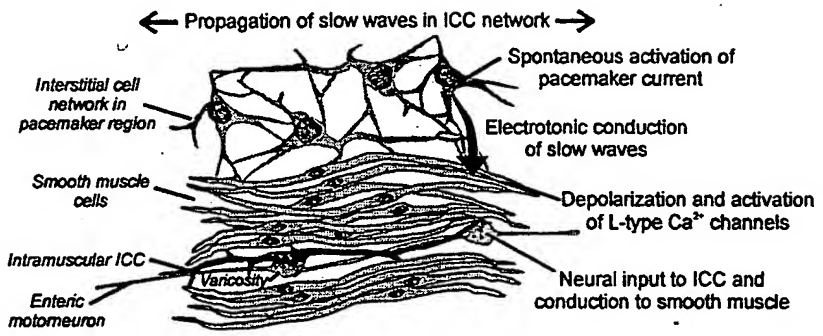


Figure 2 Model for generation and modulation of electrical activity in gastrointestinal muscles. Interstitial cell networks in pacemaker regions express the ionic mechanism to generate slow waves. These events can actively propagate through the ICC network via gap junctions connecting the ICC. Slow waves electrotonically conduct into smooth muscle cells, which are also electrically coupled to the interstitial cells of Cajal (ICC) but appear to lack the mechanism for regenerating slow waves. Slow waves depolarize the smooth muscle cells, and activate voltage-dependent (L-type) Ca^{2+} channels. If threshold depolarization is achieved, Ca^{2+} action potentials are elicited. If threshold is not achieved, the activation of inward current in smooth muscle cells is manifest as an increase in the plateau phase of slow waves. Both of these smooth muscle responses to slow wave depolarizations can be observed in the records shown in Figure 1*C* and *E*. Entry of Ca^{2+} via L-type Ca^{2+} channels in smooth muscle cells is necessary for excitation-contraction coupling. Neural inputs can condition the smooth muscle response to slow waves. Release of excitatory transmitters activates nonselective cation channels and increases the effectiveness of slow waves to bring the muscle cells to threshold. Release of inhibitory transmitters activates potassium channels, which decreases the probability of reaching threshold. Intramuscular ICC (IC-IM) are closely associated with enteric motor neurons. These cells appear to act as receivers and transducers for some of the neural inputs. Neural inputs can be transmitted through IC-IM to the smooth muscle cells because IC-IM are electrically coupled to the smooth muscle syncytium. Some neurotransmitters, particularly peptides, are likely to spill over and directly affect receptors expressed by smooth muscle cells.

completely block, rhythmicity in either intact strips of small intestinal muscle or cultured ICC. Taken together, experiments on cultured small intestinal ICC indicate that the multiple conductance pathways may be involved in the pacemaker activity of these cells.

ION CHANNEL EXPRESSION IN SMOOTH MUSCLE CELLS

Because of the relative ease in enzymatically dispersing smooth muscle cells from GI muscles, work to identify specific ionic components in these cells has progressed rapidly. Another paper in this section (32a) reviews native currents recorded from GI muscle cells. This section discusses molecular species that have been found and characterized in GI smooth muscle cells. It is important to

note that while many molecular studies have been conducted on smooth muscle tissues, with a complex tissue such as GI muscle, these studies do not determine expression of specific ionic species in particular cell types. Recent studies have begun to utilize reverse transcriptase polymerase chain reaction (RT-PCR) on identified smooth muscle cells to identify cell-specific ionic species. These results are also discussed.

Voltage-Dependent K^+ Channels

The responses of smooth muscle cells to slow wave depolarizations depend to a significant extent on the types of K^+ channels expressed. The diversity of responses observed in the various organs and regions of the GI tract also appear to depend on the complement of K^+ channels present. A variety of K^+ channel blocking drugs can dramatically affect the pattern of electrical activity in GI muscles. A search for the molecular species responsible for K^+ currents has yielded evidence for a rich diversity that helps to explain the variations in the electrical patterns observed (1).

The most diverse group of conductances in GI muscles is the voltage-dependent, Ca^{2+}-insensitive K^+ (Kv) channels. These conductances have a wide variety of pharmacologies and electrical properties. For example, Kv currents of canine colonic muscles are of the delayed rectifier type and can be separated into rapidly and slowly activating components (33). In particular, I_{dkf} is a fast-activating current blocked by micromolar concentrations of 4-aminopyridine (4-AP), I_{dks} is a slowly activating current blocked by tetraethylammonium (TEA), and I_{dkn} is a TEA-sensitive current that inactivates at negative potentials.

Mammalian genes encoding Kv channels were originally identified as homologues of the *Shaker* gene in *Drosophila* (34). The predicted topological structure of these channels is depicted in Figure 3. Organizationally, Chandy & Gutman (35) proposed to group the mammalian Kv channels into families (Kv1–4) of homologous gene products. The properties of this group of channels have been reviewed (36). In the last several years, five more families have been identified (Kv5–9) that have the curious common property of not producing functional channels when expressed singly in heterologous expression systems (37–40). These "electrically silent channels," or γ subunits (37), can have subtle effects on pharmacological as well as pore-dependent properties on coexpressed Kv channels. The expression of these subunits has not been reported in GI muscles.

Gastrointestinal smooth muscles express several Kv family members. Kv1.2 was cloned from a canine colonic smooth muscle library and detected in several other GI smooth muscles, including the stomach and small bowel (4). However, expression of cKv1.2 was not detected by Northern blot analysis in other canine smooth muscles, including renal artery, uterine wall, and portal vein. cKv1.2

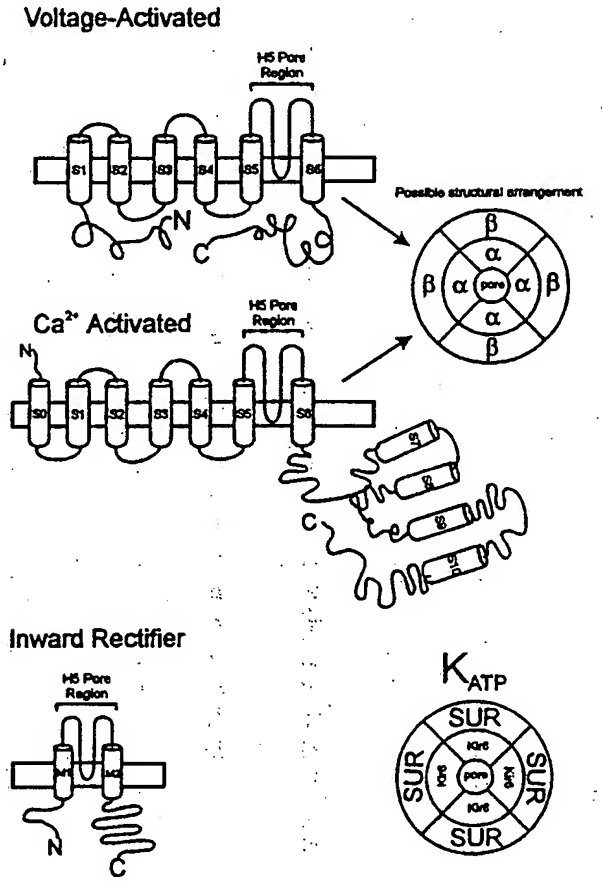


Figure 3 Predicted topological structures and hypothetical molecular arrangements of potassium channel types.

was found to be closely related to Kv1.2 genes isolated from brain libraries of other species, having only eight amino acid differences with the previously identified RCK1 from rat. (41). When expressed in *Xenopus* oocytes, cKv1.2 yielded delayed rectifier-type currents. The current activated rapidly with a half-activation time of 7.6 ± 0.2 ms and slowly inactivated. Half inactivation (V_h) occurred at −15 mV. Inactivation was incomplete (~35% of the current failed to inactivate) even with 20-s prepulses at room temperature. When the current was studied at more physiological temperatures (34°C), the activation and inactivation properties were more rapid and inactivation was more complete during a 20-s prepulse (42). cKv1.2 was found to be extremely sensitive to block by 4-AP with an IC_{50} of 74.7 μM. This sensitivity was markedly greater than

for other homologues of Kv1.2 (cloned from brain) and *Drosophila Shaker* channels. Block by 4-AP was use-dependent, and 4-AP does not appear to bind to the channels in the inactivated state.

In single-channel experiments, 4-AP decreased the mean open time of cKv1.2 channels in a dose-dependent manner but did not alter the single-channel current amplitude (42). The properties and expression pattern of cKv1.2 coordinated well with the characteristics of the fast component of the native delayed rectifier current in canine colonic myocytes (I_{dkf}) (33); however, cKv1.2 was found to be sensitive to charybdotoxin (CTX) whereas the native current was unaffected by treatment with CTX (33).

Another cDNA recovered from the same cDNA library was most closely related to Kv1.5. However, unlike cKv1.2, cKv1.5 was considerably different from other Kv1.5 members isolated from brain libraries of other species. The amino acid sequence displayed a high level of identity to other K^+ channels of the Kv1.5 class in the core region between transmembrane segments S1 and S6. However, amino acid identity decreased to between 74% and 82% in the NH_2 and COOH terminal segments, which suggests that cKv1.5 was a distinct isoform of the Kv1.5 class. Functional expression of cKv1.5 in oocytes demonstrated channels highly selective for K^+ that showed voltage-dependent activation positive to −40 mV. At room temperature, the current showed fast activation (time to half of peak current, $t_{1/2} = 5.5$ ms) and slow inactivation, which was incomplete after 20-s depolarizations. Unitary current analysis displayed a linear I-V curve with a slope conductance of 9.8 ± 1.1 pS. cKv1.5 was also sensitive to block by 4-AP with an IC_{50} of 211 μM. Northern blot analysis demonstrated differential expression of cKv1.5 in smooth muscles of the GI tract and abundant expression in several vascular smooth muscles.

Except for the discrepancy in the sensitivity of cKv1.2 to CTX, both cloned Kv channels were similar to native I_{dkf} found in canine colonic myocytes. Members of the Kv1 family form heterotetramers in heterologous expression systems (43) as well as in native cells (44). Studies of the CTX pharmacology of cKv1.2 and cKv1.5 expressed in oocytes provide evidence for heterotetramer formation in colonic myocytes (45). Even a single CTX-resistant subunit rendered the heterotetrameric channel insensitive to CTX. The two K^+-channel clones differed in an amino acid at the mouth of the pore region that may be in a position to block the access of CTX to its binding site and, hence, determine CTX sensitivity of the heterotetrameric channel. These results may explain discrepancies reported between native and cloned smooth muscle K^+ channels and suggest that native I_{dkf} in canine colonic myocytes is a Kv1.2/Kv1.5 heterotetramer.

In addition to the fast, delayed rectifier component in colonic myocytes, a slowly activating component, I_{dks}, was observed in the presence of 10 mM 4-AP. This 4-AP–insensitive component (I_{dks}), unlike I_{dkf} (33), was blocked by external tetraethylammonium (TEA). Kv2.2, homologous to the *shab* family

of *Drosophila* voltage-gated K^+ channels, was isolated from human and canine colonic circular smooth muscle–derived mRNA. DNA sequence analysis detected significant homology between the human (hKv2.2), canine (cKv2.2), and rat brain (rKv2.2) clones (46). Northern hybridization analysis performed on RNA prepared from tissues and RT-PCR performed on RNA isolated from dispersed and selected smooth muscle cells demonstrates that Kv2.2 is expressed in all regions of the canine GI tract and in several vascular tissues.

Expression of Kv2.2 mRNA in *Xenopus* oocytes resulted in a slowly activating K^+ current ($t_{1/2} = 97 \pm 8.6$ ms) mediated by 15-pS (in symmetrical K^+ gradients) channels. The current was inhibited by TEA ($IC_{50} = 2.6$ mM), 4-AP ($IC_{50} = 1.5$ mM at +20 mV), and quinine ($IC_{50} = 13.7\ \mu$M) and was insensitive to CTX. A comparison of the electrophysiological and pharmacological properties of Kv2.2 to the delayed rectifier current in native colonic smooth muscle indicates that it shares many similarities with I_{dks} and may underlie this component of the delayed rectifier K^+ current in GI smooth muscle cells. A detailed comparison of the biophysical and pharmacological characteristics of Kv clones and native currents is presented in Table 1.

An extensive analysis of the transcriptional expression of Kv channels in the canine GI tract has recently been carried out using RT-PCR performed on RNA preparations obtained from freshly dispersed and isolated smooth muscle cells (B Horowitz, unpublished observations). These experiments eliminate contamination from other cell types within GI muscles (e.g. ICC, neurons, fibroblasts, macrophages, etc) and show the presence of Kv channel types in addition to those isolated from cDNA libraries. Channels found in these studies include Kv1.4, a rapidly inactivating K^+ current (34, 47), which was found in all regions of the GI tract tested, and Kv1.6, detected in cells from the antrum, duodenum, jejunum, and colon.

Accessory subunits have been shown to associate with Kv channels to form α/β complexes (48). Three similar forms of this accessory subunit have been cloned from rat brain (β1.1, 1.2, and 2.1). These subunits affect inactivation

Table 1 Comparison of cloned Kv channels and native currents in canine colonic smooth muscle[a]

Channel	$T_{1/2}$	Activation threshold	Inactivation		IC_{50}			Channel conductance
			$V_{1/2}$	Vs	4-AP	Quinine	TEA_{out}	
cKv1.2	7.6 ms	−40 mV	−15 mV	7.7	75 μM	250 μM	<10 mM	14 pS
cKv1.5	5.5 ms	−40 mV	−21 mV	7.0	211 μM	365 μM	<10 mM	9.8 pS
$I_{dK(f)}$	7.8 ms	−40 mV	ND	ND	69 μM	ND	<10 mM	20 pS
cKv2.2	97 ms	−20 mV	−16.3 mV	4.8	1.5 mM	14 μM	2.6 mM	15.3 pS
$I_{dK(s)}$	31 ms	−20 mV	ND	ND	>1.5 mM	ND	2.2 mM	ND

[a]ND, Not determined. From Schmalz (46), reproduced with permission.

kinetics of Kv1.2 (49), Kv.1.4 (50), and Kv1.5 (51, 52). Kvβ1.1 and 1.2 are alternative splice products of the same gene (53) whereas Kvβ2 is the product of a separate gene. These β subunits interact only with Kv1 family members, but an additional accessory subunit (β4) associates with Kv2.2 (54). This interaction resulted in altered expression of Kv2.2 in heterologous systems but had no effect on the kinetics of Kv2.2. β1.1 as well as β4 were detected in all regions of the canine GI tract, but other β subunit isoforms have not been detected (46). It is cryptic that β1.1 as well as Kv1.4 would be expressed in canine GI smooth muscle. β1.1 has been shown to increase the inactivation kinetics of Kv1 family members. Kv1.4 is a rapidly inactivating channel, but fast inactivation has not been recorded from native canine colonic myocytes (33). However, a recent report demonstrated that Kv1.6 subunits can confer a dominant negative effect on the rapid inactivation conferred by Kvβ subunits and can decrease the inactivation kinetics of Kv1.4 (55). Expression of Kv1.6 by canine, mouse, and human GI smooth muscles may help to explain the discrepancy between cloned and native channels.

Differential expression of Kv channels has been related to electrical diversity in intact GI muscles. Kv conductances were characterized in canine colonic circular and longitudinal muscles. Longitudinal cells express a conductance that was TEA-sensitive and weakly affected by 4-AP (56). Treatment of intact muscles with TEA increased the amplitude and frequency of action potentials, but little effect was noted with 4-AP. In contrast, circular muscle cells of the same preparation express a significant 4-AP–sensitive current (see discussion above about proposed contributions of Kv1.2 and Kv1.5) and a TEA-sensitive component of delayed rectifier. Addition of 4-AP to intact muscles enhanced the amplitude and duration of slow waves (57). These experiments demonstrate the participation of specialized conductances in generating diverse electrical behaviors.

Regulation of Kv Channels

Muscarinic receptor stimulation enhances excitatory electrical and mechanical activity in GI muscles by increasing the amplitude and duration of slow waves (25, 58) and by increasing the probability of action potential generation (59). Muscarinic stimulation activates a nonselective cation current in GI muscles (e.g. 60), but suppression of delayed rectifier K^+ currents is another possible means of excitable regulation. With identification of the molecular components responsible for Kv channel expression in GI smooth muscles, analysis of the regulatory effects of neurotransmitters and hormones on these target proteins is possible without contamination from other conductances.

GI myocytes express both m2 and m3 receptor subtypes (61), and m3 receptors are coupled to phopholipase C (62). Functional coupling between m3 receptors, Kv1.2, and Kv1.5 was studied by using *Xenopus* oocytes and COS

cells coinjected with cRNAs encoding human m3 receptors and Kv channel clones (63). Acetylcholine decreased whole cell Kv currents in these cells, and phorbol esters [e.g. phorbol dibutyrate (PDBu)] mimicked the action of acetylcholine (ACh). At the single-channel level, ACh and PDBu applied to the extra-patch membrane reduced the open probability of Kv channels in cell-attached patches without affecting single-channel conductance. It is difficult to extrapolate the function of this regulatory pathway to native smooth muscle cells, but the reconstitution experiments demonstrate the utility of a molecular approach in dissecting signal transduction mechanisms in GI smooth muscle cells.

Inhibitory neural regulation of GI muscles also acts via Kv-like channels, but molecular equivalents to these events have not been determined. Carbon monoxide (CO) increased whole cell outward currents, hyperpolarized membrane potential, and increased guanosine 3′,5′-cyclic monophosphate (cGMP) levels in canine jejunal smooth muscle cells (64). Heme oxygenase 2, the enzyme responsible for producing CO, has been localized to ICC cells in the murine small intestine, which suggests that CO could be an endogenous regulator of outward currents (65). Nitric oxide (NO) also has been found to activate Kv-like channels in native GI smooth muscle cells. NO and NO donors increased the open probabilities of 80-pS and 4-pS voltage-dependent K^+ channels in canine colonic myocytes (66). The molecular identification of these channels is not known. VIP (vasoactive intestinal peptide) can enhance delayed rectifier whole cell currents in native canine colon myocytes, probably via a cAMP-dependent mechanism (67). The currents activated were 4-AP sensitive and likely to be a component of I_{dk} assigned to Kv1.2/1.5; however, this type of regulation has not been examined on specific molecular targets.

Calcium-Activated K^+ Channels

The initial report of a cDNA encoding Ca^{2+}-activated K^+ channels (BK channels) was from studies of *Drosophila*, and the gene was termed *slowpoke* or *dslo* (68). Several homologues of the *Drosophila* cDNA have been isolated from mammalian brains (69, 70). A human vascular muscle homologue was also isolated (71). The topological structure of these K^+ channels is similar to Kv channels in the core domain (S1–S6) (see Figure 3) (72); however, *slo* gene products have an extensive COOH terminal tail, which possesses a "calcium bowl" domain that may impart the Ca^{2+} sensitivity to the channel (73). When injected into *Xenopus* oocytes, the *slowpoke* cRNA expressed Ca^{2+}-activated, voltage-sensitive K^+ channels with many of the electrophysiological and pharmacological properties of native channels (74). Significant differences, however, were noted between Ca^{2+} sensitivities of expressed and native channels. These differences could be due to species variability (70), diversity in

alternative spice variants (75), or the presence of accessory subunits (76). A β subunit was cloned from tracheal smooth muscle (77) and shown to enhance the Ca^{2+} sensitivity of expressed channels (76). These authors observed a 10-fold increase in Ca^{2+} sensitivity when α and β subunits were coexpressed. Studies on the *dslo* channel cDNA have demonstrated that cloned channels can be modulated by protein kinase A and that phosphorylation of the *dslo* channel protein itself was responsible (78).

Both α and β subunits of Ca^{2+}-activated K^+ channels were cloned from canine colonic smooth muscle (79). This was the first report showing the cloning and expression of both subunits from the same tissue. A splice variant of the α subunit with diversity in the carboxy terminal region was also recovered. Northern analysis demonstrated expression of both α and β subunits in all canine vascular and visceral smooth muscles tested. Expression of α alone and $\alpha + \beta$ subunits in *Xenopus* oocytes resulted in Ca^{2+} and voltage-dependent conductances. The $\alpha + \beta$ channels were more sensitive to [Ca^{2+}]-free medium than were channels composed of α subunit alone, and the $\alpha + \beta$ channels more closely resembled the Ca^{2+} sensitivity of native BK channels in colonic smooth muscle. This suggests that native BK channels are composed of α and β subunits. Despite the enhanced Ca^{2+} sensitivity of $\alpha + \beta$ channels versus α channels, native BK channels are considerably more sensitive to Ca^{2+} than are cloned channels. For example, the voltage of half-maximal activation ($V_{0.5}$) of native BK channels at [Ca^{2+}] = 1 μM were approximately 0 mV (80), but for $\alpha\beta$ cloned channels $V_{0.5}$ lies in the positive range (+50 mV). The reasons for these differences between cloned and native channels is not currently understood. It should be noted, however, that cloned α channels from myometrium expressed with bovine tracheal β subunits have similar Ca^{2+} sensitivity to native myometrial channels (81).

The role of BK channels in GI electrical activity is far from clear. Although Ca^{2+} entry would tend to enhance the open probability of these channels, the negative membrane potentials at which GI muscles operate are far from optimal for channel activation. Calculations from single-channels studies in which the Ca^{2+} and voltage dependence of this conductance were characterized suggests that NP_0 was negligible at the resting potential and basal intracellular Ca^{2+} concentration of canine colonic circular smooth muscles (see 80). Application of CTX to intact colonic muscle had little or no effect on basal electrical activity (33); however, when muscles were excited with ACh, evidence was obtained for recruitment of BK channels. Thus, in some GI muscles, BK channels may be utilized as a break on excitatory stimuli. In other regions of the GI tract with more positive resting potentials and a tendency for action potential generation, BK channels may play an ongoing role in regulation of electrical activity (e.g. 33, 82).

Currents from small-conductance, Ca^{2+}-activated K^+ channels have been recorded in murine smooth muscle cells from ileum and colon, and these channels are activated by purinergic stimulation (83, 84). Apamin sensitive and insensitive components of this current were present. The molecular equivalent of these currents in GI smooth muscles have not been identified; however, currents with similar properties in mouse and human brain are encoded by members of the SK family (85). The gene products of the SK family (SK1–3) have a typical S1–S6 topological structure, including the positively charged amino acids in the S4 transmembrane segment characteristic of voltage-gated K^+ channels (85). However, the channels are not voltage dependent. Expression of SK channels resulted in Ca^{2+}-activated (0.6–0.7 μM K_d Ca^{2+}), small-conductance (9–10 pS in symetrical K^+ solutions), voltage-insensitive K^+ channels. Although SK2 was highly sensitive to apamin, SK1 showed no sensitivity. These channels can form heteromeric structures that may affect their pharmacology (86). These channels are important in GI motility because they mediate a portion of the inhibitory response to nerve stimulation.

Inward Rectifier and ATP-Sensitive K^+ Channels

Identified members of the inward rectifier gene family have expanded over the last several years, and this family now includes six subfamilies based on DNA sequence homologies (87). To date, three distinct isoforms of the Kir2 channel subfamily have been identified in the rat brain: Kir2.1 (88), Kir2.2 (89), and Kir2.3 (90). Members of the Kir2 family show strong inward rectification and possess consensus sites for phosphorylation by protein kinases A and C (PKA and PKC, respectively) (91).

Application of Ba^{2+} (1–100 μM) caused concentration-dependent depolarization of resting membrane potential in canine colon circular muscle (ERM Flynn, CA McManus, SD Koh, KM Sanders, submitted). Whole cell patch clamp studies on isolated canine colonic myocytes detected small, inwardly rectifying, Ba^{2+}-sensitive K^+ currents, indicating the presence of Kir2-like currents (SI Cho, KM Sanders, submitted). Molecular evidence demonstrated that Kir2.1 was expressed in cells from canine colon muscle as well as other canine GI smooth muscles (B Horowitz, unpublished data). Expression in oocytes and mammalian cells resulted in a strongly inwardly rectifying, Ba^{2+}-sensitive K^+ channel. Other members of the Kir2 family have not been detected in GI tissues.

Members of the Kir3 family encode G-protein–gated, inward-rectifying K^+ channels (GIRKs) (88, 94). In heart muscle, activation of a potassium current by muscarinic stimulation (I_{KACh}) mediated a slowing of heart rate (95, 96). Krapivinsky et al (97) identified the molecular components underlying this current as a combination of Kir3.1 and Kir3.4. However, other combinations of Kir3 family members can encode a similar current. In the GI tract, this

type of K^+ current may act to assist repolarization between slow waves during muscarinic responses, thus preserving phasic electrical and mechanical activity. Kir3.1 expression in canine colon was found to be quantitatively similar to β-actin (i.e. high expression), and Kir3.1 can be detected in RT-PCR on RNA prepared from isolated cells (B Horowitz, unpublished data). Kir3.2 and 3.3 but not 3.4 were detectable in canine colonic myocytes, and combinations of Kir3.1 and other Kir3 family members can form heterotetrameric channels when expressed in oocytes (97).

ATP-dependent potassium channels (K_{ATP}) produce weakly inwardly rectifying K^+ selective currents that are modulated by the metabolic state of cells (98). Their physiological importance is well established in the vasculature, but less is known about the physiological significance of this conductance in GI smooth muscles. K_{ATP} channel agonists, such as cromakalim and nicorandil, have been shown to hyperpolarize and relax stomach (99), ileum (100), and colon (101, 102).

K_{ATP} channels are formed from association of a sulfonylurea receptor (SUR) and an inward-rectifier K^+ channel of the Kir6 family (103–105). Two separate genes have been identified that encode the SUR component (SUR1 and 2). Alternative splicing arrangements exist for the SUR 2 isoform that can affect the pharmacology of the resulting K_{ATP} channel. SUR2A and 2B differ in their carboxy terminal amino acid sequence. SUR2A/Kir6.2 resulted in a "cardiac-like" K_{ATP} current that is inhibited by >100 μM ATP, and the sulfonylurea glibenclamide was stimulated by pinacidil and cromakalim but not by diazoxide. SUR2B/Kir6.1 has been proposed to be the molecular equivalent of a vascular smooth muscle K_{ATP} current (105, 106). Expression of this combination in oocytes resulted in a channel that is stimulated at lower concentrations of ATP (0.1–100 μM), inhibited by higher concentrations (1–3 mM) of ATP, and activated by both pinacidil and diazoxide. These characteristics are similar to the K_{NDP} channel identified in smooth muscle (107–109). Recently, the SUR2 gene has been shown to be alternatively spliced at exon 14 and exon 17, and these variants displayed subtly altered pharmacologies (110). An analysis of mouse colon RNA using RT-PCR detected the expression of SUR2B (but not SUR1 or SUR2A) and Kir6.1 (but not 6.2) (106). However, the cellular origin of these transcripts was not made clear from this report. A recent study using RT-PCR on dispersed and isolated mouse colon smooth muscle cells (111) identified Kir6.2 (but not 6.1), in contrast to Isomoto et al (106), and SUR2B (but not SUR1 or SUR2A), confirming the report from Isomoto et al (106).

Ca^{2+} Channels

The predominant Ca^{2+} channels in smooth muscle are dihydropyridine-sensitive or L-type channels. L-type Ca^{2+} channels provide the Ca^{2+} influx that initiates

contraction (e.g. 112, 113). Blockade of Ca^{2+} channels reduces the duration and amplitude of electrical slow waves in many muscles and blocks generation of action potentials. Blockade of L-type Ca^{2+} channels does not block slow waves, which suggests that other inward current sources are available in ICC and needed for these events (see discussion above).

L-type Ca^{2+} channels are composed of five subunits, $\alpha_1\beta\gamma\alpha_2\delta$, of which the α_1 subunit contains the ion permeation pathway (cf 114–116). The auxiliary $\beta\gamma\alpha_2\delta$ subunits modulate channel activity and channel kinetics of activation and inactivation. Several separate genes encode the conducting α_1 subunit, and there are also splice variants of these α_1 genes (117). The α_1 subtype in canine colonic smooth muscle has been identified as $\alpha_{1C\text{-}b}$ (118). However, identification of the auxiliary subunits expressed in GI smooth muscle has not been performed. In human jejunum smooth muscle, the predominant Ca^{2+} current is dihydropyridine sensitive. This current is carried by 17-pS (in 80 mM Ba^{2+}) barium-permeable channels (119). This current was found to be regulated by G-proteins (120) and has many properties in common with the $\alpha_{1C\text{-}b}$ channel identified in canine myocytes.

Cl^- Channels

The role of chloride channels in GI motility has not been studied as extensively as the functions of K^+ or Ca^{2+} channels. However, in other smooth muscles, chloride conductances are important regulators of electrical excitability (121–124). Chloride currents contribute to cell volume regulation (125), cardiac action potential modulation (126), and transepithelial transport (127). Macroscopic chloride currents recorded from native cells have either a linear or an outwardly rectifying current-voltage relationship; however, some cloned Cl^- channels show either inwardly rectifying or more complex biophysical properties when expressed in heterologous systems (128). Regulation of Cl^- currents is diverse and β adrenergic (126) and α adrenergic pathways have been shown to be involved (129). In addition, chloride channels modulated by Ca^{2+} (130), ATP (through purinergic stimulation), and cell volume or mechanical stretch (131) have been recorded. Finally, in some cells a basal chloride conductance that contributes to resting membrane potential has been reported (132).

The gene products encoding Cl^- channels include GABA receptors, the cystic fibrosis transmembrane conductance regulator (CFTR), and the ClC gene family. CFTR is likely to encode the β adrenergic–regulated Cl^- current ($I_{Cl.camp}$) and may also be responsible for Cl^- currents regulated by ATP and PKC (activated through α adrenergic stimulation). CFTR is expressed in a variety of cell types, although GI smooth muscles have yet to be examined. The molecular equivalent of a niflumic acid–sensitive, Ca^{2+}-activated Cl^- conductance ($I_{Cl.Ca}$) has not been identified, and the role of this conductance in GI smooth muscles

is unclear, although evidence for $I_{Cl.Ca}$ in ICC from the murine small intestine has been reported (31). Recently, a swelling-activated Cl^- conductance ($I_{Cl.swell}$) that may also be responsible for a basal Cl^- current ($I_{Cl.b}$) in some cell types has been cloned (ClC-3) (131, 133). A swelling-activated Cl^- current with properties identical to ClC-3 has been observed in GI myocytes. This current is activated under isotonic conditions ($I_{Cl.b}$) and sensitive to PKC stimulation (134). Niflumic acid had no effect on the swelling-activated current, which suggests this current is distinct from Ca^{2+}-activated Cl^- conductance ($I_{Cl.Ca}$). ClC-3 is expressed in GI myocytes and is likely to encode the swelling-activated current. PKC activation inhibited ClC-3 and has a similar effect on $I_{Cl.swell}$ and $I_{Cl.b}$ in smooth muscle cells. Therefore, this current could be an important component of the ACh response in this tissue.

CONCLUSIONS

Electrical rhythmicity in the gut helps to organize the phasic contractions that are essential for normal GI motility. Determination of the molecular entities responsible for the ionic mechanisms of rhythmicity will provide us with a clear understanding of the basic processes underlying motility. Identification of cloned ion channel genes and association of the gene products with native currents in GI smooth muscles lies at the heart of understanding the mechanisms of rhythmicity. Molecular studies are now contributing significantly to the discovery of ionic conductances not previously described in GI muscles and providing a powerful means of studying the regulation of these channels. The precise contribution of specific molecular entities is complex and will continue to evolve. The novel suggestions in this review regarding the role of ICC in generating pacemaker activity and propagating slow waves and the distinct roles of ICC and smooth muscle cells in the electrical activity of the GI tract should alter the focus of investigations into the mechanisms of rhythmicity and emphasize the need for cell-specific identification of molecular species. As we advance with cell-specific identification of ion channels and as the molecular structures of channels are more completely understood, including the role of chaperone proteins (135) and accessory subunits, the list of molecules involved in producing, propagating, and regulating electrical activity in the gut will undoubtedly grow.

ACKNOWLEDGMENTS

Research in our laboratories was supported by a Program Project Grant (DK41315) and Research Grants (HL52803 to BH and DK 40569 to KMS). We would like to acknowledge the work of all the investigators involved in the PPG and their individual laboratories.

Literature Cited

1. Szurszewski JH. 1987. Electrical basis for gastrointestinal motility. In *Physiology of the Gastrointestinal Tract*, pp. 383–422. New York: Raven. 2nd ed.
2. Bolton TB, Lang RJ, Takewaki T, Benham CD. 1985. Patch and whole-cell voltage clamp of single mammalian visceral and vascular smooth muscle cells. *Experientia* 41:887–94
3. Mitra R, Morad M. 1985. Ca^{2+} and Ca^{2+}-activated K^+ currents in mammalian gastric smooth muscle cells. *Science* 229: 269–72
4. Hart PJ, Overturf KE, Russell SN, Carl A, Hume JR, et al. 1993. Cloning and expression of a $K_v1.2$ class delayed rectifier K^+ channel from canine colonic smooth muscle. *Proc. Natl. Acad. Sci. USA* 90:9659–63
5. Overturf KE, Russell SN, Carl A, Vogalis F, Hart PJ, et al. 1994. Cloning and characterization of a $K_v1.5$ delayed rectifier K^+ channel from vascular and visceral smooth muscles. *Am. J. Physiol.* 267:C1231–38
6. Sanders KM. 1996. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* 111:492–515
7. Bauer AJ, Sanders KM. 1986. Passive and active membrane properties of canine gastric antral circular muscles. *Am. J. Physiol.* 251:C268–73
8. Smith TK, Reed JB, Sanders KM. 1987. Interaction of two electrical pacemakers in muscularis of canine proximal colon. *Am. J. Physiol.* 252:C290–99
9. Smith TK, Reed JB, Sanders KM. 1987. Origin and propogation of electrical slow waves in circular muscle of canine proximal colon. *Am. J. Physiol.* 252:C215–24
10. Hara Y, Kubota M, Szurszewski JH. 1986. Electrophysiology of smooth muscle of the small intestine of some mammals. *J. Physiol.* 372:501–20
11. Xiong Z, Sperelakis N, Noffsinger A, Fenoglio-Preiser C. 1993. Changes in calcium channel current densities in rat colonic smooth muscle cells during development and aging. *Am. J. Physiol.* 265: C617–25
12. Thuneberg L. 1982. Interstitial cells of Cajal: intestinal pacemaker cells. *Adv. Anat. Embryol. Cell Biol.* 71:1–130
13. Christensen JA. 1992. Commentary on the morphological identification of interstitial cells of Cajal in the gut. *J. Auton. Nerv. Syst.* 37:75–88
14. Daniel EE, Berezin I. 1992. Interstial cells of Cajal: are they major players in control of gastrointestinal motility? *J. Gastrointest. Motil.* 4:1–24
15. Daniel EE, Posey-Daniel V. 1984. Neuromuscular structures in opossum esophagus: role of interstitial cells of Cajal. *Am. J. Physiol.* 246:G305–15
16. Huizinga JD, Thuneberg L, Vanderwinden JM, Rumessen JJ. 1997. Interstitial cells of Cajal as targets for pharmacological intervention in gastrointestinal motor disorders. *Trends Pharmacol. Sci.* 18:393–403
17. Langton P, Ward SM, Carl A, Norell MA, Sanders KM. 1989. Spontaneous electrical activity of interstitial cells of Cajal isolated from canine proximal colon. *Proc. Natl. Acad. Sci. USA* 86:7280–84
18. Ward SM, Burns AJ, Torihashi S, Sanders KM. 1994. Mutation of the proto-oncogene c-*kit* blocks development of interstitial cells and electrical rhythmicity in murine intestine. *J. Physiol.* 480:91–97
19. Huizinga JD, Thuneberg L, Kluppel M, Malysz J, Mikkelsen HB, Bernstein A. 1995. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 373:347–49
20. Torihashi S, Ward SM, Nishikawa S-I, Nishi K, Kobayashi S, Sanders KM. 1995. c-*kit*-dependent development of interstitial cells and electrical activity in the murine gastrointestinal tract. *Cell Tissue Res.* 280:97–111
21. Burns AJ, Lomax AEJ, Torihashi S, Sanders KM, Ward SM. 1996. Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc. Natl. Acad. Sci. USA* 93:12008–13
22. Prosser CL. 1978. Rhythmic potentials in intestinal muscle. *Fed. Proc.* 37:2153–57
23. Suzuki N, Prosser CL, Dahms V. 1986. Boundary cells between longitudinal and circular layers: essential for electrical slow waves in cat intestine. *Am. J. Physiol.* 250:G287–94

24. Liu LW, Huizinga JD. 1994. Canine colonic circular muscle generates action potentials without the pacemaker component. *Can. J. Physiol. Pharmacol.* 72:70–81
25. Keef KD, Ward SM, Stevens RJ, Frey BW, Sanders KM. 1992. Electrical and mechanical effects of acetylcholine and substance P in subregions of canine colon. *Am. J. Physiol.* 262:G298–307
26. Sanders KM, Burke EP, Carl A, Cole WC, Langton P, Ward S. 1990. Mechanism of electrical rhythmicity in colonic smooth muscle: an hypothesis. *Prog. Clin. Biol. Res.* 327:307–22
27. Ward SM, Burns AJ, Torihashi S, Harney SC, Sanders KM. 1995. Impaired development of interstitial cells and intestinal electrical rhythmicity in *steel* mutants. *Am. J. Physiol.* 269:C1577–85
28. Sanders KM, Publicover NG. 1989. Electrophysiology of the gastric musculature. In *Handbook of Physiology: The Gastrointestinal System*, ed. S Schultz, JD Wood, pp. 187–216. Bethesda, MD: Am. Physiol. Soc.
29. Lee HK, Sanders KM. 1993. Comparison of ionic currents from interstitial cells and smooth muscle cells of canine colon. *J. Physiol.* 460:135–52
30. Hagiwara N, Irisawa H, Kameyama M. 1988. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. *J. Physiol.* 395:233–53
31. Tokutomi N, Maeda H, Tokutomi Y, Sato D, Sugita M, et al. 1995. Rhythmic Cl^- current and physiological roles of the intestinal c-kit-positive cells. *Pflügers Arch.* 431:169–77
32. Malysz J, Richardson D, Farraway L, Christen MO, Huizinga JD. 1995. Generation of slow wave type action potentials in the mouse small intestine involves a non-L-type calcium channel. *Can. J. Physiol. Pharmacol.* 73:1502–11
32a. Faruggia G. 1999. Ionic conductances in gastrointestinal smooth muscles and interstitial cells of Cajal. *Annu. Rev. Physiol.* 61:45–84
33. Carl A. 1995. Multiple components of delayed rectifier K^+ current in canine colonic myocytes. *J. Physiol.* 484:339–53
34. Stuhmer W, Ruppersberg JP, Schroter KH, Sakmann B, Stocker M, et al. 1989. Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. *EMBO J.* 8:3235–44
35. Chandy KG, Gutman GA. 1993. Nomenclature for mammalian potassium channel genes. *Trends Pharmacol. Sci.* 14:434
36. Chandy KG, Gutman GA. 1995. Voltage-gated K^+ channel genes. In *Ligand and Voltage-Gated Ion Channels*, ed. A North, pp. 1–71. Boca Raton, FL: CRC
37. Patel AJ, Lazdunski M, Honore E. 1997. Kv2.1/Kv9.3, a novel ATP-dependent delayed-rectifier K^+ channel in oxygen-sensitive pulmonary artery myocytes. *EMBO J.* 16:6615–25
38. Hugnot JP, Salinas M, Lesage F, Guillemare E, De Weille J, et al. 1996. Kv8.1, a new neuronal potassium channel subunit with specific inhibitory properties towards Shab and Shaw channels. *EMBO J.* 15:3322–31
39. Salinas M, De Weille J, Guillemare E, Lazdunski M, Hugnot JP. 1997. Modes of regulation of shab K^+ channel activity by the Kv8.1 subunit. *J. Biol. Chem.* 272:8774–80
40. Salinas M, Duprat F, Heurteaux C, Hugnot JP, Lazdunski M. 1997. New modulatory alpha subunits for mammalian Shab K^+ channels. *J. Biol. Chem.* 272:24371–79
41. Beckh S, Pongs O. 1990. Members of the RCK potassium channel family are differentially expressed in the rat nervous system. *EMBO J.* 9:777–82
42. Russell SN, Publicover NG, Hart PJ, Carl A, Hume JR, et al. 1994. Block by 4-aminopyridine of a $K_v1.2$ delayed rectifier K^+ current expressed in *Xenopus* oocytes. *J. Physiol.* 481:571–84
43. Ruppersberg JP, Schroter KH, Sakmann B, Stocker M, Sewing S, Pongs O. 1990. Heteromultimeric channels formed by rat brain potassium-channel proteins. [see Comments] *Nature* 345:535–37
44. Wang H, Kunkel DD, Martin TM, Schwartzkroin PA, Tempel BL. 1993. Heteromultimeric K^+ channels in terminal and juxtaparanodal regions of neurons. *Nature* 365:75–79
45. Russell SN, Overturf KE, Horowitz B. 1994. Heterotetramer formation and charybdotoxin sensitivity of two K^+ channels cloned from smooth muscle. *Am. J. Physiol.* 267:C1729–33
46. Schmalz F, Kinsella JL, Koh SD, Vogalis F, Schneider A, Flynn ERM, et al. 1998. Molecular identification of a component of delayed rectifier current in gastrointestinal smooth muscles. *Am. J. Physiol.* 274:G901–11
47. Po S, Snyders DJ, Baker R, Tamkun MM, Bennett PB. 1992. Functional expression of an inactivating potassium channel

cloned from human heart. *Circ. Res.* 71: 732–36
48. Scott VE, Rettig J, Parcej DN, Keen JN, Findlay JB, et al. 1994. Primary structure of a beta subunit of alpha-dendrotoxin-sensitive K^+ channels from bovine brain. *Proc. Natl. Acad. Sci. USA* 91:1637–41
49. Rettig J, Heinemann SH, Wunder F, Lorra C, Parcej DN, et al. 1994. Inactivation properties of voltage-gated K^+ channels altered by presence of beta-subunit. *Nature* 369:289–94
50. McIntosh P, Southan AP, Akhtar S, Sidera C, Ushkaryov Y, et al. 1997. Modification of rat brain Kv1.4 channel gating by association with accessory Kvβ1.1 and β2.1 subunits. *Pflügers Arch.* 435:43–54
51. England SK, Uebele VN, Shear H, Kodali J, Bennett PB, Tamkun MM. 1995. Characterization of a voltage-gated K^+ channel β subunit expressed in human heart. *Proc. Natl. Acad. Sci. USA* 92:6309–13
52. Sewing S, Roeper J, Pongs O. 1996. Kv beta 1 subunit binding specific for shaker-related potassium channel alpha subunits. *Neuron* 16:455–63
53. McCormack K, McCormack T, Tanouye M, Rudy B, Stühmer W. 1995. Alternative splicing of the human *Shaker* K^+ channel β1 gene and functional expression of the β2 gene product. *FEBS Lett.* 370:32–36
54. Fink M, Duprat F, Lesage F, Heurteaux C, Romey G, et al. 1996. A new K^+ channel β subunit to specifically enhance Kv2.2 (CDRK) expression. *J. Biol. Chem.* 271:26341–48
55. Roeper J, Sewing S, Zhang Y, Sommer T, Wanner SG, Pongs O. 1998. NIP domain prevents N-type inactivation in voltage-gated potassium channels. *Nature* 391:390–93
56. Thornbury KD, Ward SM, Sanders KM. 1992. Outward currents in longitudinal colonic muscle cells contribute to spiking electrical behavior. *Am. J. Physiol.* 263:C237–45
57. Thornbury KD, Ward SM, Sanders KM. 1992. Participation of fast-activating voltage-dependent K currents in electrical slow waves of colonic circular muscle. *Am. J. Physiol.* 263:C226–36
58. Szurszewski JH. 1975. Mechanism of action of pentagastrin and acetylcholine on the longitudinal muscle of the canine antrum. *J. Physiol.* 252:335–61
59. Sanders KM. 1983. Excitation-contraction coupling without Ca^{2+} action potentials in small intestine. *Am. J. Physiol.* 244:C356–61
60. Inoue R, Chen S. 1993. Physiology of muscarinic receptor-operated nonselective cation channels in guinea-pig ileal smooth muscle. *Experimentia* 66:261–68
61. Zhang LB, Horowitz B, Buxton IL. 1991. Muscarinic receptors in canine colonic circular smooth muscle. I. Coexistence of M2 and M3 subtypes *Mol. Pharmacology* 40:943–51
62. Lechleiter J, Hellmiss R, Duerson K, Ennulat D, David N, et al. 1990. Distinct sequence elements control the specificity of G protein activation by muscarinic acetylcholine receptor subtypes. *EMBO J.* 9:4381–90
63. Vogalis F, Ward M, Horowitz B. 1995. Suppression of two cloned smooth muscle-derived delayed rectifier potassium channels by cholinergic agonists and phorbol esters. *Mol. Pharmacol.* 48: 1015–23
64. Farrugia G, Miller SM, Rich A, Liu X, Maines MD, et al. 1998. Distribution of heme oxygenase and effects of exogenous carbon monoxide in canine jejunum. *Am. J. Physiol.* 274:G350–58
65. Miller SM, Farrugia G, Schmalz PF, Ermilov LG, Maines MD, Szurszewski JH. 1998. Heme oxygenase 2 is present in interstitial cell networks of the mouse small intestine. *Gastroenterology* 114:239–44
66. Koh SD, Campbell JD, Carl A, Sanders KM. 1995. Nitric oxide activates multiple potassium channels in canine colonic smooth muscle. *J. Physiol.* 489:735–43
67. Shuttleworth CWR, Koh SD, Bayginov O, Sanders KM. 1996. Activation of delayed rectifier potassium channels in canine proximal colon by vasoactive intestinal peptide. *J. Physiol.* 493:651–63
68. Atkinson NS, Robertson GA, Ganetzky B. 1991. A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science* 253: 551–55
69. Pallanck L, Ganetzky B. 1994. Cloning and characterization of human and mouse homologs of the *Drosophila* calcium-activated potassium channel gene *slowpoke*. *Hum. Mol. Genet.* 3:1239–43
70. Tseng-Crank J, Foster CD, Krause JD, Mertz R, Godinot N, et al. 1994. Cloning expression and distribution of functionally distinct Ca^{2+}-activated K^+ channel isoforms from human brain. *Neuron* 13:1315–30
71. McCobb DP, Fowler NL, Featherstone T, Lingle CJ, Saito M, et al. 1995. A human calcium-activated potassium channel gene expressed in vascular smooth muscle. *Am. J. Physiol.* 269:H767–77

72. Meera P, Wallner M, Song M, Toro L. 1997. Large conductance voltage- and calcium-dependent K^+ channel a distinct member of voltage-dependent ion channels with seven N-terminal transmembrane segments (SO-S6), an extracellular N terminus and an intracellular (S9–S10) C terminus. *Proc. Natl. Acad. Sci. USA* 94:14066–71
73. Schreiber M, Salkoff L. 1997. A novel calcium-sensing domain in the BK channel. *Biophys. J.* 73:1355–63
74. Adelman JP, Shen K-Z, Kavanaugh MP, Warren RA, Wu Y-N, et al. 1992. Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron* 9:209–16
75. Lagrutta A, Shen K-Z, North RA, Adelman JP. 1994. Functional differences among alternatively spliced variants of *Slowpoke* a *Drosophila* calcium-activated potassium channel. *J. Biol. Chem.* 269: 20347–51
76. McManus OB, Helms LMH, Pallanck L, Ganetzky B, Swanson R, Leonard RJ. 1995. Functional role of the β subunit of high conductance calcium-activated potassium channels. *Neuron* 14:645–50
77. Knaus HG, Folander K, Garcia-Calvo M, Garcia ML, Kaczorowski GJ, et al. 1994. Primary sequence and immunological characterization of beta-subunit of high conductance Ca^{2+}-activated K^+ channel from smooth muscle. *J. Biol. Chem.* 269:17274–78
78. Esguerra M, Wang J, Foster CD, Adelman JP, North RA, Levitan IB. 1994. Cloned Ca^{2+}-dependent K^+ channel modulated by a functionally associated protein kinase. *Nature* 369:563–65
79. Vogalis F, Vincent T, Qureshi I, Schmalz F, Ward MW, et al. 1996. Cloning and expression of the large-conductance Ca^{2+}-activated K^+ channel from colonic smooth muscle. *Am. J. Physiol.* 271: G629–39
80. Carl A, Sanders KM. 1989. Ca^{2+}-activated potassium channels of canine colonic myocytes. *Am. J. Physiol.* 257: C470–80
81. Wallner M, Meera P, Ottolia M, Kacsorowski GJ, Latorre R, et al. 1995. Characterization of and modulation by a beta-subunit of a human maxi KCa channel cloned from myometrium. *Recept. Channels* 3:185–99
82. Hong SJ, Roan YF, Chang CC. 1997. Spontaneous activity of guinea pig ileum longitudinal muscle regulated by Ca^{2+}-activated K^+ channel. *Am. J. Physiol.* 272: G962–71
83. Vogalis F, Goyal RK. 1997. Activation of small conductance Ca^{2+}-dependent K^+ channels by purinergic agonists in smooth muscle cells of the mouse ileum. *J. Physiol.* 502:497–508
84. Koh SD, Dick GM, Sanders KM. 1997. Small-conductance Ca^{2+}-dependent K^+ channels activated by ATP in murine colonic smooth muscle. *Am. J. Physiol.* 273:C2010–21
85. Kohler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, et al. 1996. Small-conductance calcium-activated potassium channels from mammalian brain. [see Comments] *Science* 273:1709–14
86. Ishii TM, Maylie J, Adelman JP. 1997. Determinants of apamin and d-tubocurarine block in SK potassium channels. *J. Biol. Chem.* 272:23195–200
87. Doupnik CA, Davidson N, Lester HA. 1995. The inward rectifier potassium channel family. *Curr. Opin. Neurobiol.* 5: 268–77
88. Kubo Y, Baldwin TJ, Jan YN, Jan LY. 1993. Primary structure and functional expression of a mouse inward rectifier potassium channel. *Nature* 362:127–33
89. Koyama H, Morishige K-I, Takahashi N, Zanelli JS, Fass DN, Kurachi Y. 1994. Molecular cloning functional expression and localization of a novel inward rectifier potassium channel in the rat brain. *FEBS Lett.* 341:303–7
90. Morishige K-I, Takahashi N, Jahangir A, Yamada M, Koyama H, et al. 1994. Molecular cloning and functional expression of a novel brain-specific inward rectifier potassium channel. *FEBS Lett.* 346:251–56
91. Henry P, Pearson WL, Nichols CG. 1996. Protein kinase C inhibition of cloned inward rectifier (HRK1/KIR2.3) K^+ channels expressed in Xenopus oocytes. *J. Physiol.* 495:681–88
92. Deleted in proof
93. Deleted in proof
94. Dascal N. 1997. Signalling via the G protein-activated K^+ channels. *Cell. Signal* 9:551–73
95. Kurachi Y, Nakajima T, Sugimoto T. 1986. Acetylcholine activation of K^+ channels in cell-free membrane of atrial cells. *Am. J. Physiol.* 251:H681–84
96. Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE. 1987. The beta gamma subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. *Nature* 325:321–26

97. Krapivinsky G, Gordon EA, Wickman K, Velimirovic B, Krapivinsky L, Clapham DE. 1995. The G-protein-gated atrial K^+ channel I_{KACh} is a heteromultimer of two inwardly rectifying K^+-channel proteins. *Nature* 374:135–41
98. Quayle JM, Nelson MT, Standen NB. 1997. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol. Rev.* 77:1165–232
99. Katayama N, Huang SM, Tomita T, Brading AF. 1993. Effects of cromakalim on the electrical slow wave in the circular muscle of guinea-pig gastric antrum. *Br. J. Pharmacol.* 109:1097–100
100. Franck H, Puschmann A, Allescher HD. 1994. Functional evidence for a glibenclamide-sensitive K+ channel in rat ileal smooth muscle. *Eur. J. Pharmacol.* 271:379–86
101. Faraway L, Huizinga JD. 1991. Potassium channel activation by cromakalim affects the slow wave type action potential of colonic smooth muscle. *J. Pharmacol. Exp. Ther.* 257:35–41
102. Post JM, Stevens R, Sanders KM, Hume JR. 1991. Effect of cromakalim and lemakalim on K^+ and Ca^{2+} currents in colonic smooth muscle. *Am. J. Physiol.* 260:C375–82
103. Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, et al. 1995. Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. [see Comments] *Science* 270:1166–70
104. Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, et al. 1996. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* 16:1011–17
105. Yokoshiki H, Sunagawa M, Seki T, Sperelakis N. 1998. ATP-sensitive K^+ channels in pancreatic cardiac and vascular smooth muscle cells. *Am. J. Physiol.* 274:C25–37
106. Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, et al. 1996. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J. Biol. Chem.* 271: 24321–24
107. Kajioka S, Kitamura K, Kuriyama H. 1991. Guanosine diphosphate activates an adenosine 5′-triphosphate-sensitive K^+ channel in the rabbit portal vein. *J. Physiol.* 444:397–418
108. Zhang H, Bolton TB. 1995. Activation by intracellular GDP metabolic inhibition and pinacidil of a glibenclamide-sensitive K-channel in smooth muscle cells of rat mesenteric artery. *Br. J. Pharmacol.* 114:662–72
109. Zhang HL, Bolton TB. 1996. Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells. *Br. J. Pharmacol.* 118:105–14
110. Fan Z, Chutkow WA, McClelland DL, Burant CF, Makielski JC. 1998. Nucleotide gating of ATP-sensitive K channels formed by variants of SUR1 and SUR2 isoforms co-expressed with Kir6.2. *Biophys. J.* 74:A18 (Abstr.)
111. Koh SD, Kuenzli KA, Rae M, Keef K, Horowitz B, Sanders KM. 1998. Basal activation of ATP-sensitive potassium channels in murine colonic smooth muscle cells. *Biophys. J.* In press
112. Ozaki H, Gerthoffer WT, Publicover NG, Fusetani N, Sanders KM. 1991. Time-dependent changes in Ca^{2+} sensitivity during phasic contraction of canine antral smooth muscle. *J. Physiol.* 440:207–24
113. Vogalis F, Publicover NG, Hume JR, Sanders KM. 1991. Relationship between calcium current and cytosolic calcium in canine gastric smooth muscle cells. *Am. J. Physiol.* 260:C1012–18
114. Catterall WA. 1996. Molecular properties of sodium and calcium channels. *J. Bioenerg. Biomembr.* 28:219–30
115. Perez-Reyes E, Schneider T. 1995. Molecular biology of calcium channels. *Kidney Int.* 48:1111–24
116. Mori Y, Mikala G, Varadi G, Kobayashi T, Koch S, et al. 1996. Molecular pharmacology of voltage-dependent calcium channels. *Jpn. J. Pharmacol.* 72:83–109
117. Birnbaumer L, Campbell KP, Catterall WA, Harpold MM, Hofmann F, et al. 1994. The naming of voltage-gated calcium channels. *Neuron* 13:505–6
118. Rich A, Kenyon JL, Hume JR, Overturf K, Horowitz B, Sanders KM. 1993. Dihydropyridine-sensitive calcium channels expressed in canine colonic smooth muscle cells. *Am. J. Physiol.* 264:C745–54
119. Farrugia G, Rich A, Rae JL, Sarr MG, Szurszewski JH. 1995. Calcium currents in human and canine jejunal circular smooth muscle cells. *Gastroenterology* 109:707–17
120. Farrugia G. 1997. G-protein regulation of an L-type calcium channel current in canine jejunal circular smooth muscle. *J. Membr. Biol.* 160:39–46
121. Criddle DN, de MR, Greenwood IA, Large WA. 1997. Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor

responses in the isolated mesenteric vascular bed of the rat. *Br. J. Pharmacol.* 120:813–18
122. Greenwood IA, Helliwell RM, Large WA. 1997. Modulation of Ca^{2+}-activated Cl^- currents in rabbit portal vein smooth muscle by an inhibitor of mitochondrial Ca^{2+} uptake. *J Physiol.* 505:53–54
123. Criddle DN, de MR, Greenwood IA, Large WA. 1996. Effect of niflumic acid on noradrenaline-induced contractions of the rat aorta. *Br. J. Pharmacol.* 118:1065–71
124. Nelson MT, Conway MA, Knot HJ, Brayden JE. 1997. Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. *J. Physiol.* 502:259–64
125. Okada Y. 1997. Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. *Am. J Physiol.* 273:C755–89
126. Harvey RD, Hume JR. 1989. Autonomic regulation of a chloride current in heart. *Science* 244:983–85
127. Carroll TP, Schwiebert EM, Guggino WB. 1993. CFTR: structure and function. *Cell. Physiol. Biochem.* 3:388–99
128. Jentsch TJ, Günther W, Pusch M, Schwappach B. 1995. Properties of voltage-gated chloride channels of the ClC gene family. *J. Physiol.* 482(Suppl. P):19–25S
129. Coca-Prados M, Anguita J, Chalfant ML, Civan MM. 1995. PKC-sensitive Cl^- channels associated with ciliary epithelial homologue of pICln. *Am. J Physiol.* 268:C572–79
130. Large WA, Wang Q. 1996. Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. *Am. J. Physiol.* 271:C435–54
131. Duan D, Winter C, Cowley S, Hume JR, Horowitz B. 1997. Molecular identification of a volume-regulated chloride channel. *Nature* 390:417–21
132. Duan D, Hume JR, Nattel S. 1997. Evidence that outwardly rectifying Cl^- channels underlie volume-regulated Cl^- currents in heart. *Circ. Res.* 80:103–13
133. Yamazaki J, Duan D, Janiak R, Kuenzli K, Horowitz B, Hume JR. 1998. Functional and molecular expression of volume-regulated chloride channels in canine vascular smooth muscle cells. *J. Physiol.* 507: 729–36
134. Dick GM, Kuenzli KA, Horowitz B, Hume JR, Sanders KM. 1998. Cl^- current activated by cell swelling in visceral smooth muscle. *Biophys. J.* 74:A99 (Abstr.)
135. Xia X, Hirschberg B, Smolik S, Forte M, Adelman JP. 1998. dSLo interacting protein 1, a novel protein that interacts with large-conductance calcium-activated potassium channels. *J. Neurosci.* 18:2360–69
136. Sanders KM, Burke EP, Carl A, Cole WC, Langton P, Ward SM. 1990. *Frontiers in Smooth Muscle Research*. New York: Liss